World *Pyrus* Collection at USDA Genebank in Corvallis, Oregon

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Abstract

In 1980 the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), established a genebank, the National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. This facility is devoted to conservation of temperate fruit and nut crops. A globally diverse collection of *Pyrus* germplasm has been assembled and expanded over the subsequent 27 years. Unique pear genotypes are maintained as growing plants, evaluated for phenotypic and genotypic traits, tested for virus contamination, documented in a national public germplasm database, and freely distributed to international researchers. Seed collections represent wild *Pyrus* populations. The Corvallis genebank now maintains 2031 clonal *Pyrus* accessions and 327 seedlots representing 36 taxa from 53 countries. About 10% of clonal accessions are backed up either in vitro or as cryopreserved meristems. A DNA microsatellite fingerprint database has been initiated. About 75% of the clonal collection has tested negative for common latent viruses. During the past 3 years, on average 775 accessions were distributed annually to researchers around the world. While originally conceived as a working collection for crop improvement, this genebank also provides the raw materials for basic genetic research, and preserves rare species, vulnerable landraces and historic cultivars. The NCGR collection serves as a laboratory and a classroom to promote the preservation of pear genetic diversity for future generations.

INTRODUCTION

Beginning in 1980, the USDA-ARS established 8 genebanks to preserve genetic resources of important fruit and nut crops. The mission includes acquisition, documentation, preservation, evaluation, enhancement, and distribution of plant genetic resources (Postman et al., 2006). The National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, maintains collections of more than 26 genera including *Actinidia*, *Corylus*, *Cydonia, Fragaria*, *Pyrus*, *Ribes, Rubus*, and *Vaccinium*. The NCGR *Pyrus* collection (Table 1) was initially assembled in a 5 hectare orchard located at Oregon State University by repropagating trees from several existing U.S. pear collections. The clonal pear accessions were grafted onto the disease resistant OHxF 333 rootstock (Hummer, 1994). In addition to pear clones, NCGR preserves pear species populations as seedlots stored at -20°C. In 1994 the NCGR genebank contained 1389 clonal accessions and 70 seed accessions (Hummer, 1994). Since 1994, genetic and taxonomic gaps in the collection have been identified (Bell et al., 1996; NPGS, 2004) and significant efforts made to acquire pear germplasm to fill these gaps. Recent expeditions to Armenia and Georgia have added wild-collected samples of *P. communis* ssp. *caucasica* and *P. salicifolia* from their center of origin in the trans-Caucasus region (NPGS, 2004).

NPGS Pear Germplasm Collection 2007

Accessions added to the NCGR collection in recent years have expanded the representation of world *Pyrus* germplasm at this USDA genebank. The collection presently includes 2031 clonal accessions and 327 seedlots representing 36 taxa from 53 countries (Tables 1 and 2). The clonal collection is represented by 1781 trees in 5 hectares

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of orchard plantings, with a single tree per accession. Clonal accessions include 844 European cultivars, 144 Asian cultivars, 87 hybrid cultivars and 159 rootstock selections of assorted *Pyrus* species.

Clonal Backup Collections

Species such as *Pyrus koenhei* or *P. pashia* that originate in sub-tropical regions may not be reliably cold hardy in Corvallis, Oregon at 44.6° north latitude. Potted backup trees are maintained in a glasshouse for 48 clonal accessions of these and other non-hardy species. In vitro culture of growing shoots stored at 1 to 4°C is used as a medium-term backup for 10% of the clonal collection, and cryogenic storage of apical meristems is used for long-term backup of about 100 clones (Reed et al., 2004).

Seed and Species Collections

The 327 seed accessions at NCGR include 176 samples with fewer than 100 seed. While 70% of *Pyrus* seedlots have viability >50%, some have low viability as determined by triphenyl tetrazolium chloride staining assay (Peters, 2000). Seedlings have been generated from many low-number or low-viability seedlots, and from other seedlots of taxa poorly represented in the collection. A new field plot was established in 2005 with up to 5 seedlings per seedlot, and divided into separate blocks for 1) West European and North African pear species; 2) Middle East and West Asia species; 3) East Asia "pea pear" species and 4) larger fruited East Asia species. These seedling populations will be evaluated for unique traits, and controlled crosses will be made within populations to regenerate seedlots.

Pathogen Testing

Clonal pear accessions are tested for common germplasm borne viruses by graft-bioassay to sensitive indicator clones. More than 75% of the cultivar accessions at NCGR are available as virus tested plants, and 173 clones are only available as virus infected plants. A pear clone is considered to be "tested" if it has tested negative in greenhouse bioassays using the two graft inoculated indicator clones *P. communis* 'Nouveau Poiteau' and ×*Pyronia veitchii* (Trab.) Guillaumin which together will detect apple stem pitting virus (=pear vein yellows), apple chlorotic leafspot virus (=pear ringpattern mosaic) and sometimes apple stem grooving virus. Additional greenhouse, field and laboratory assays are also used. Nearly 400 virus tested trees were generated at NCGR Corvallis using heat therapy and meristem culture to clean up previously virus infected clones. Many of these are not available elsewhere as virus tested trees (Postman and Sugar, 2002).

Molecular Fingerprints

Microsatellite or simple sequence repeat (SSR) markers developed at NCGR from GenBank pear sequences (Bassil et al., 2005) or developed by others for apple or pear (Yamamoto et al., 2002) have proven to be polymorphic in a diverse group of pear accessions at NCGR. These markers are being used to generate unique fingerprints that will enable us to quickly locate sources of unique traits, identify unknown pear cultivars and eliminate duplication in the collection. The markers have recently been used to compare the identities of old pear trees in the Azores and in two U.S. National Parks to known cultivars at NCGR (Bassil et al., 2006).

Pear Germplasm Information Online

NCGR Corvallis has provided online access to its catalogs since the early days of the internet (Hummer and Sugar, 1998). The Germplasm Resources Information Network or GRIN is the national public database that provides both gene bank personnel and germplasm user's access to passport, characterization, evaluation, inventory, and distribution data from the national germplasm collections (NPGS, 2006). Updated NCGR catalogs, links to pear accession images, evaluation data and other useful pear genetic resource information is available at the genebank website (Postman and Hummer, 2006).

Germplasm Distribution and Exchange

Since NCGR-Corvallis was established in 1980, it has received requests for 12,665 clonal pear samples, and 446 seed samples from researchers around the world. Between the years 2000 and 2006 distribution of pear seeds, scions and other samples has increased from under 500 to more than 900 samples annually with scions representing the majority of samples distributed (Fig. 1). A survey of the most requested accessions reveals that after 'Bartlett' and 'Bosc', several perry cultivars and high quality Asian cultivars have been in high demand. The most requested seed samples include cold-hardy species with potential use as rootstocks (Table 3).

NCGR Pear Collection in the Future

Although originally planned as a working collection in support of crop improvement, the NCGR pear genebank also provides raw materials for basic genetic research, and preserves rare species and historic cultivars. At least 250 cultivars are known to be over 100 years old, and many are much older (Hummer, 1994). Continuing efforts to eliminate unintended redundancy, fill genetic gaps, and expand evaluation data in GRIN will enhance the value of the NPGS *Pyrus* collection for future generations.

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Tables

Table 1. USDA National Plant Germplasm System *Pyrus* holdings by species (NPGS, 2007a).

Location	Count
Site	
National Clonal Germplasm Repository, Corvallis, Oregon	2209
Plant Germplasm Quarantine Program, Beltsville, Maryland	151
Taxon	
Pyrus amygdaliformis Vill.	38
Pyrus betulifolia Bunge	73
Pyrus boissieriana Buhse	1
Pyrus calleryana Decne.	125
Pyrus communis L.	1001
Pyrus communis ssp. *caucasica* (Fed.) Browicz	66
Pyrus communis ssp. *pyraster* (L.) Ehrh.	44
Pyrus complexa Rubtzov	2
Pyrus cordata Desv.	24
Pyrus cossonii Rehder	4
Pyrus dimorphophylla Makino	26
Pyrus elaeagrifolia Pall.	33
Pyrus elaeagrifolia ssp. *kotschyana* (Boiss. ex Decne.) Browicz	1
Pyrus fauriei C.K.Schneid.	36
Pyrus gharbiana Trab.	8
Pyrus glabra Boiss.	1
Pyrus hondoensis Nakai & Kikuchi	44
Pyrus hybrid	194
Pyrus koehnei C.K. Schneid.	17
Pyrus korshinskyi Litv.	5
Pyrus korshinskyi ssp. *bucharica* (Litv.) Bondarenko ex Korovina	1
Pyrus mamorensis Trab.	23
Pyrus nivalis Jacq.	21
Pyrus pashia Buch.-Ham. ex D.Don	34
Pyrus pseudopashia T.T. Yu	2
Pyrus pyrifolia (Burm. f.) Nakai	121
Pyrus regelii Rehder	11
Pyrus sachokiana Kuth.	3
Pyrus salicifolia Pall.	35
Pyrus spp.	249
Pyrus syriaca Boiss.	14
Pyrus ussuriensis Maxim.	90
Pyrus ×*bretschneideri* Rehder	4
Pyrus ×*canescens* Spach	1
Pyrus ×*phaeocarpa* Rehder	2
Pyrus ×*serrulata* Rehder	3
Pyrus ×*uyematsuana* Makino	1
Pyrus xerophila T.T. Yu	3

Table 2. USDA National Plant Germplasm System *Pyrus* holdings by origin (NPGS, 2007b).

Country	Accessions	Species	Country	Accessions	Species
Afghanistan	3	3	Morocco	6	3
Albania	7	3	Nepal	15	4
Armenia	50	7	Netherlands	8	3
Asia	2	2	Norway	1	1
Australia	21	6	Pakistan	37	5
Belgium	51	1	Poland	24	5
Bulgaria	8	2	Portugal	3	1
Canada	43	6	Romania	34	4
China	118	11	Russia	63	14
Czech Republic	29	6	South Africa	10	2
Denmark	3	1	Spain	2	1
Estonia	10	1	Sweden	6	2
Former Sov.Union	27	5	Switzerland	6	2
France	181	7	Syria	4	1
Georgia	37	4	Taiwan	4	1
Germany	15	1	Tajikistan	1	1
Greece	1	1	Tunisia	7	1
Hungary	8	4	Turkey	49	5
India	30	4	Turkmenistan	16	1
Iran	3	2	Ukraine	3	1
Israel	3	3	United Kingdom	88	10
Italy	77	5	United States	917	29
Japan	70	6	Unknown	1	1
Kazakhstan	18	6	Uzbekistan	17	11
Korea, South	21	4	Vietnam	1	1
Kyrgyzstan	3	1	Yugoslavia	42	5
Macedonia	31	4	no data available	126	19

Table 3a. Twenty most requested of 2011 *Pyrus* clones at NCGR Corvallis from 1981 to 2007.

Requests	Plant name	Type
110	Bartlett	European Cultivar
104	Bosc (OP-5 clone)	European Cultivar
98	Tse Li	Asian Cultivar
97	Ya Li	Asian Cultivar
85	Seckel	European Cultivar
83	Kosui	Asian Cultivar
81	Red Pear	European Perry Cultivar
74	Nijisseiki	Asian Cultivar
73	Kalle (=Red Clapp Favorite)	European Cultivar
71	Yellow Huffcap	European Perry Cultivar
70	Thorn	European Perry Cultivar
66	Magness	European Cultivar
65	Shinseiki	Asian Cultivar
64	Barland	European Perry Cultivar
64	Gin	European Perry Cultivar
63	Blakeney Red	European Perry Cultivar
63	Butirra Precoce Morettini	European Cultivar
60	Le Nain Vert (Genetic Dwarf)	European Cultivar
59	Duchesse d'Angouleme	European Cultivar
58	Butt	European Perry Cultivar

Table 3b. Twelve most requested of 327 *Pyrus* seed accessions.

Requests	Plant name
23	*P. betulifolia* – cold hardy from Shaanxi, China (PYR 2291)
14	*P. betulifolia* (PYR 2033)
12	*P. elaeagrifolia* (PYR 1054)
11	*P. amygdaliformis* – from Macedonia (PYR 330)
10	*P. calleryana* D-6 x Bradford Dwarf (PYR 1029)
9	*P. koehnei* – from Taiwan (PYR 962)
9	*P. regelii* – from Kazakhstan (PYR 2554)
9	*P. ussuriensis* 'McDermand' – from Harbin, China (PYR 1501)
8	*P. betulifolia* No. 1 (PYR 755)
7	*P. amygdaliformis* – from Macedonia (PYR 104)
7	*P. betulifolia* – from China (PYR 1699)
7	*P. communis* ssp. *pyraster* – from Turkey (PYR 67)

Figures

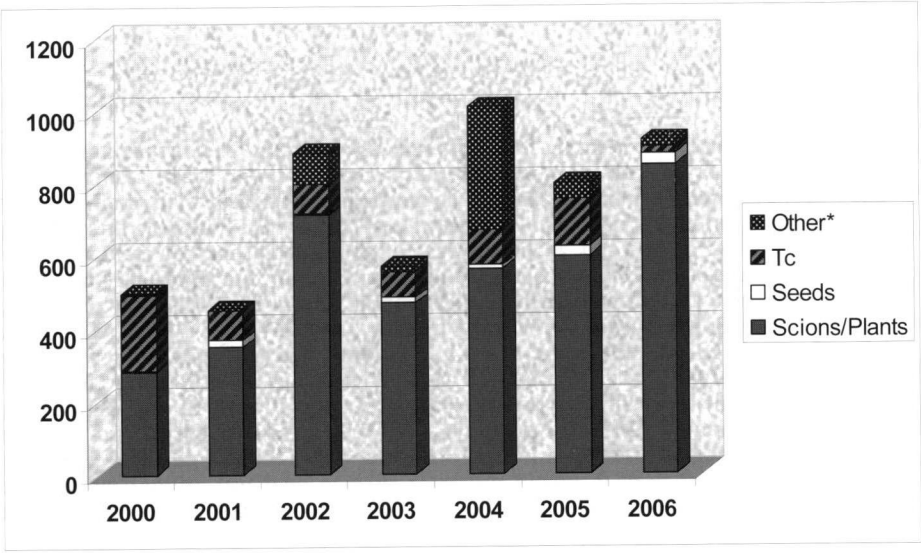

Fig. 1. USDA National Plant Germplasm System *Pyrus* distributions 2000–2006 (NPGS, 2007c). *'Other' includes DNA, leaves, pollen, fruit, etc.